claims pending with this response is provided in Appendix II. Reconsideration of the application in view of the above amendments and following remarks is respectfully requested.

GENERAL REMARKS ON AMENDMENTS TO THE CLAIMS

Claim 21 has been amended to more clearly define Applicant's invention: a PCR based method for detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample using a set of at least 5 oligonucleotide primer pairs, at least one primer pair for each of 5 subgroups, wherein detecting the presence of at least one amplified product indicates the presence of at least one subgroup in the sample. Dependent Claims 22 and 23 are drawn to the method of Claim 21, reciting specific embodiments of the primer pairs. Dependent Claim 24 is drawn to the method of Claim 21, wherein the detection of amplified product is performed using a labeled oligonucleotide probe. Claims 26 and 27 are dependent on Claim 24 and recite specific embodiments of the labeled oligonucleotide probes. Dependent Claim 37-40 are drawn to the method of Claim 21, reciting specific uses for the method.

Claim 30 has been amended to more clearly recite Applicant's invention: a set of oligonucleotide primer pairs useful for a PCR only based method of detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, wherein the set consists of at least 5 primer pairs, at least one pair for each subgroup. Dependent Claims 31 and 32 are drawn to the set of primer pairs of Claim 30, reciting specific embodiments of the primer pairs.

Claim 33 has also been amended to more clearly recite Applicant's invention. Amended Claim 33 is drawn to a set of labeled, oligonucleotide probes useful for a Real-Time PCR based method of detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye, at the 3' end with a fluorescent quencher dye, and are susceptible to 5'-3' exonuclease degradation by a polymerase, wherein the set consists of at least one probe for each subgroup. Dependent Claims 34 and 35 are drawn to the set of labeled, oligonucleotide probes of Claim 33, reciting specific embodiments of the probes.

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Amended Claim 36 is drawn to a set of primer pairs AND probes useful for a Real-Time PCR based method of detection and differentiation of pathogenic enterobacteria in a sample.

SUPPORT FOR AMENDMENTS TO THE CLAIMS

Support for the amendments can be found throughout the specification as filed. For example, support for "isolating nucleic acid from said sample" can be found at, e.g., page 23, line 24 to page 24, line 6; page 25, line 24; page 27, first paragraph. Support for a set of oligonucleotide primer pairs consisting of at least 5 primer pairs can be found at, e.g., page 25, lines 25-26 and Table II. Support for "wherein the presence of at least one amplified product indicates the presence of at least one pathogenic E. coli strain in said sample" can be found at, e.g., Example 1, especially the first paragraph of page 8. Support for "wherein the detection of amplified product is performed using a labeled oligonucleotide probe" can be found at, e.g., Example 2, especially paragraphs b and c.

Claims 22 and 31 have been amended to more clearly recite possible, alternative embodiments of the at least 5 oligonucleotide primer pairs, e.g., at least one of the primer pairs is a primer pair that hybridizes to a gene encoding heat stabile toxin or a primer pair that hybridizes to a pCDVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative E. coli. Support for the amendments to Claims 22 and 31 can be found throughout the specification as filed.

Claims 26 and 34 have been amended to more clearly recite possible embodiments of the labeled, oligonucleotide probes, e.g., the set comprises a probe specific for at least a heat labile toxin gene or a heat stabile toxin gene characteristic for enterotoxigenic E. Coli, etc.. Support for the amendments to Claims 26 and 34 can be found throughout the specification as filed.

Amendments to Claim 33 are supported throughout the specification as filed, e.g., support for "wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and are susceptible to 5'-3' exonuclease degradation by a polymerase," can be found at, e.g., page 22, second paragraph.

Claim 36 is amended in a manner similar to Claim 30, e.g., the set of oligonucleotide primer pairs consists of at least 5 primer pairs. Claim 36 is also amended to correct inadvertent and/or typographical errors.

Claims 38 and 40 are amended to correct inadvertent and/or typographical errors, e.g., to correct dependency. Claim 38 correctly depends on Claim 37 and not 21; Claim 40 correctly depends on Claim 39 and not 29.

The amendments to the claims therefore add no new matter.

IDS

Applicant notes with appreciation the Examiner's thorough consideration of the references cited in the IDS (Form 1449) submitted on March 14, 2002. The Examiner indicated that the reference to the BLAST search was lined through due to improper citation, as the 1449 did not indicate the date the search was performed or what was searched. Applicant intends to file a second IDS with a correct citation to the BLAST search.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 21-29 and 36-40 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner stated that:

> Claims 21-29 and 37-40 are indefinite because the preamble of claim 1 recites "A Polymerase Chain Reaction (PCR) method for detection and differentiation of pathogenic enterobacteria in a sample," but the claim does not contain any process steps which require PCR. The claims require a step of "subjecting said sample and said set of primer pairs to an amplification process," but never particularly require a PCR step as set forth in the preamble. Thus, it is not clear if the claims are meant to require a PCR step or any amplification step that would utilize oligonucleotide primers. Furthermore, the claim never specifically sets forth a method step in which a pathogenic enterobacteria is detected in a sample. The final process step of claim 21 merely sets forth the detection of

> "amplified product" but does not set forth the relationship between the amplified product and the preamble of the claim which recites that the method is for the detection and differentiation of enterobacteria.

Applicant has amended Claim 21 to recite the process steps of amplification including: a step of isolating nucleic acid from the sample, an amplification step that utilizes the oligonucleotide primer pairs, a detection step, and a relationship between the amplified product and detection and differentiation of pathogenic enterobacteria. Claims 22-29 and 37-40 are dependent on Claim 21 and therefore include the base claim limitations.

The Examiner also stated that

> Claims 24-28 and 38 are further indefinite over the recitation of "further comprising an oligonucleotide probe..." because the claim never sets forth a method step that utilizes or employs the probe, thus it is not clear what it means that the method "comprises" the probe.

Applicant has amended Claim 24 to replace the language "further comprising an oligonucleotide probe" with "wherein detecting the presence of at least one amplified product is performed using at least one oligonucleotide probe." These amendments more clearly recite Applicant's invention as a PCR based method for detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample using a set of at least 5 oligonucleotide primer pairs, at least one primer pair for each subgroup, wherein detecting the presence of at least one amplified product indicates the presence of at least one subgroup in the sample (Claim 21), and wherein detection of amplified product is performed using labeled oligonucleotide probes (dependent Claim 24).

The Examiner also stated that:

> Furthermore, claim 24 recites that the method comprises only a single probe, yet claim 21 requires, at the very least, the amplification of at least two DNA sequences, each specific to a different subgroup of E. coli. Therefore, it is unclear if claim 24 intends for only one probe to be included in the method or more than one probe. Claims 25-28 and 38 are indefinite for these same

> reasons because they depend from claim 24 and do not remedy the cited deficiencies of claim 24.

Applicant has amended Claim 21 to recite a method using a set of oligonucleotide primer pairs consisting of at least 5 primer pairs, and has amended claim 24 to recite "detecting the presence of at least one amplified product using at least one oligonucleotide probe." Thus Claim 24 clearly requires at least one oligonucleotide probe, e.g., requires Real-Time PR detection of at least one amplified product using at least one labeled oligonucleotide probe.

The Examiner also stated that:

> Claim 26 is indefinite because it appears to require that the same labeled oligonucleotide probe be specific for the detection of nine different characteristics, since each section of the claim recites "the labeled oligonucleotide probe" and there is no designation in the claim that these probes are meant to be alternatives. Furthermore, it is noted that none of the previous claims require the amplification of target DNA for each and every one of the "characteristics" listed in claim 26. Claim 27 is indefinite insomuch as it depends from claim 26 and it is not clear how many or which probes are required for use in the recited methods.

Applicant has amended Claim 26 to recite language indicating that the probes are meant to be alternatives, e.g., Claim 26 now includes the language "wherein the oligonucleotide probe is selected from the group consisting of." As already discussed above, Claims 21 and 24 have also been amended to recite amplification of at least 5 target DNAs (Claim 21) and detection of at least one target DNA using a labeled, oligonucleotide probe (Claim 24).

The Examiner also stated that:

> In claim 28, the recitation "fluorescent reporter dye" lacks proper antecedent basis because neither claim 21 nor claim 24 from which claim 28 depends specifically recites a "fluorescent reporter dye."

Applicant has amended claim 24 to include the language "fluorescent reporter dye."

The Examiner also stated that:

> Claims 36 is indefinite over the recitation of "is included in said set of oligonucleotide probes" in line 9 of the claim because the previous description in the claim is directed to the set of oligonucleotide primers, and thus the recitation of "in said set of oligonucleotide probes" is confusing. Claim 36 is further confusing because line 9 recites "and a set of oligonucleotide probes." This recitation is confusing because the first line of the claim recites "A set of primer pairs and set of oligonucleotide primer probes" and it is not clear if the set of "oligonucleotide probes" recited in line 9 of the claim is the set of oligonucleotide primer probes recited in the preamble or a different set of oligonucleotide probes.

Applicant has amended Claim 36 to more clearly recite the invention by deleting additional language that was added by inadvertent typographical error.

Applicant notes that Claim 25 is cancelled; Claim 38 has been amended to correctly depend from Claim 37 and not 27, rendering the above rejections to these claims moot.

Applicant believes that the above described amendments to the claims overcome the 112, second paragraph rejection to the claims, and respectfully requests withdrawal of these rejections.

REJECTIONS UNDER 35 U.S.C. § 102

Claims 21 and 22 are rejected under 35 U.S.C. 102(b) as allegedly anticipated by Lang et al (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149). Applicant traverses this ground of rejection by amendment and argument: the reference does not teach each and every element of the claimed invention, as required for anticipation.

Amended Claim 21 is directed to a PCR based method for detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample using a set of at least 5 oligonucleotide primer pairs, at least one primer pair for each of 5 subgroups, wherein detection of amplified product indicates the presence of at least one of the 5 pathogenic enterobacteria in the sample.

Claim 22, reciting embodiments of genes to which the primer pairs can hybridize, is dependent on Claim 21 and includes all the base claim limitations.

Lang et al fails to teach each and every element of amended Claim 21. Lang et al teaches a PCR based method for detection and differentiation of enterotoxigenic or enterohemorrhagic E. coli strains. Lang et al fails to teach a method capable of detecting 5 pathogenic enterobacteria in a sample; Lang et al only teaches detection of 2 subgroups. Therefore Lang et al fails to teach each and every element of Claim 21 or dependent Claim 22. Applicant respectfully requests withdrawal of this rejection.

Claim 13 is rejected under 35 U.S.C. 102(b) as allegedly anticipated by Levine et al. (American Journal of Epidemiology (1993 Nov 15) 138(10)849-869). As there is no pending claim 13, Applicant argues against this rejection to **Claim 33**. Applicant traverses this ground of rejection by amendment and by argument: the reference does not teach each and every element of the claimed invention, as required for anticipation.

Claim 33 was directed to a set of oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real-Time PCR. As is well known to one of skill in the art, in order for the oligonucleotide to be useful in Real-Time PCR, the probe must be short enough to not compete with the PCR primer for binding sites; purified and free from contaminating DNA fragments; and labeled with a fluorescent reporter dye and a fluorescent quencher dye. Accordingly, all of the oligonucleotide probes in the set recited in Claim 33 must meet these requirements.

Levine et al. fails to teach each and every element of Claim 33. Levine et al teaches a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample. However, the set of probes taught by Levine et al includes one labeled oligonucleotide probe and several labeled, cloned fragments of double stranded DNA. These cloned fragments are NOT useful for Real-Time PCR for the following reasons: the cloned fragments are long enough to

compete with primer biding sites; the cloned fragments are not free from impurities; the cloned fragments are not labeled with a fluorescent reporter dye and a fluorescent quencher dye.

Therefore, Applicant maintains the previously presented argument that Levine et al fails to teach each and every element of Claim 33. However, in order to expedite prosecution, Applicant has amended Claim 33 to include the language "wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and are susceptible to 5'-3' exonuclease degradation by a polymerase." Nowhere does Levine et al teach a set of labeled oligonucleotide probes that are labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and are susceptible to 5'-3' exonuclease degradation by a polymerase. Therefore Levine et al fails to teach each and every element of amended Claim 33. Applicant respectfully requests withdrawal of this rejection.

REJECTIONS UNDER 35 U.S.C. § 103

Claims 21, 22, 23, and 29 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Lang et al. (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149) in view of Yamamoto et al. (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733) and further in view of Hogan et al. (US 5595874). Applicant traverses this ground of rejection by amendment and argument: the combination of art does not teach all of the elements of the claims.

Amended Claim 21 is drawn to a PCR based method for detection of 5 subgroups of pathogenic enterobacteria in a sample using at least 5 primer pairs, at least one primer pair for each subgroup, wherein detection of amplified product indicates the presence of at least one subgroup in the sample. Amended Claims 22 and 23 are dependent on Claim 21, and recite alternative embodiments of the 5 primer pairs. Claim 29 is dependent on Claim 21 and recites specific PCR amplification conditions. Therefore, claims 21, 22, 23, and 29 all include the elements of simultaneous detection of 5 subgroups, a set of at least 5 primer pairs, and at least

one primer pair for each subgroup. The combination of art cited by the Examiner does not include these elements, and therefore cannot render these claims obvious.

As noted above, Lang et al teaches a PCR based method for detection and differentiation of enterotoxigenic or enterohemorrhagic E. coli strains. Lang et al fails to teach a method capable of detecting 5 pathogenic enterobacteria in a sample; Lang et al only teaches detection of 2 subgroups. Lang et al also fails to teach a method that uses at least one primer pair for detection of each subgroup; Lang et al uses two sets of oligonucleotide primer pairs (to the sltI and sltII genes) to reliably detect and differentiate the presence of enterohemorrhagic E. coli.

Neither Yamamoto et al nor Hogan et al remedies these deficiencies of Lang et al. Yamamoto merely teaches a nucleotide sequence of heat labile toxin of an E. coli pathogenic for humans. Hogan et al teaches primer selection. Neither prior art reference teaches a method capable of detecting 5 subgroups of pathogenic enterobacteria in a sample, using at least 5 primer pairs, or using at least one primer pair for detection of each subgroup. Therefore, the combination of art does teach not every element of claims 21, 22, 23, and 29 and the combination cannot render the claim obvious.

Claims 21, 22, 24, 25, 26 and 28 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Lang et al. (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149) in view of Livak et al. (PCR Methods and Applications (1995) 4:357-362). Applicant traverses this ground of rejection by amendment and argument: the combination of art does not teach all of the elements of the claims.

Amended Claim 21 is drawn to a PCR based method for detection of 5 subgroups of pathogenic enterobacteria in a sample using at least 5 primer pairs, at least one primer pair for each subgroup, wherein detection of amplified product indicates the presence of at least one subgroup in the sample. Amended Claim 22 is dependent on Claim 21 and recites alternative embodiments of the 5 primer pairs. Dependent Claim 24 is drawn to the method of Claim 21, wherein the detection of amplified product is performed using a labeled oligonucleotide probe.

Claims 26 and 28 are dependent on Claim 24 and recite alternative embodiments of the labeled oligonucleotide probe (Claim 26) and specific embodiments of the fluorescent dyes (Claim 28). Claim 25 has been cancelled. Therefore, claims 21, 22, 24, 25, 26 and 28 all include the elements of simultaneous detection of 5 subgroups, a set of at least 5 primer pairs, and at least one primer pair for each subgroup. The combination of art cited by the Examiner does not include these elements: as noted above Lang et al does not include these elements, and Livak et al (teaching Real-Time PCR) does not remedy the deficiencies of Lang et al. Therefore the combination of art cannot render the claims obvious.

Claim 27 is rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Lang et al. in view of Livak et al. as applied to claims 21, 22, 24, 25, 26 and 28 above, and further in view of both Yamamoto et al. and Hogan et al. Applicant traverses this ground of rejection by amendment and argument: the combination of art does not teach all of the elements of the claims.

Amended Claim 27, dependent on claims 24 and then 21, is drawn to a PCR based method for detection of 5 subgroups of pathogenic enterobacteria in a sample using at least 5 primer pairs, at least one primer pair for each subgroup, wherein detection of amplified product indicates the presence of at least one subgroup in the sample, wherein the detection of amplified product is performed using a labeled oligonucleotide probe, reciting specific oligonucleotide probe alternatives. The combination of art cited by the Examiner does not include all of the elements of claim 27: as noted above, neither Lang et al, Livak et al., Yamamoto et al. nor Hogan et al include simultaneous detection of 5 subgroups, a set of at least 5 primer pairs, and at least one primer pair for each subgroup. The combination of art does not contain all elements. Therefore the combination of art cannot render the claims obvious.

Claims 30, 33, 36, and 37 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Levine et al. (American Journal of Epidemiology, Nov. 15, 1993, 138(10): 849-869) in view of Lang et al. (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149). Applicant traverses this ground of rejection by amendment and argument: the

combination of art does not teach all of the elements of the claims; further, one of skill would have no expectation of success.

The cited prior art combination does not teach all elements of the claims. Amended Claim 30 is directed to a set of oligonucleotide primer pairs useful for a PCR based method of detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample, wherein the set consists of at least 5 primer pairs, at least one pair for each subgroup; amended Claim 33 is drawn to a set of labeled, oligonucleotide probes useful for a Real-Time PCR based method of detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye, at the 3' end with a fluorescent quencher dye, and are susceptible to 5'-3' exonuclease degradation by a polymerase, wherein the set consists of at least one probe for each of the 5 subgroups; Claim 36 is directed to a set of primer pairs (similar to those of Claim 30) together with a set of labeled oligonucleotide probes (similar to those of Claim 33); Claim 37 is dependent on method claim 21 wherein the method is used to diagnose an enterobacteria infection in a living animal body.

The rejected claims all include two elements: a set of at least 5 oligonucleotide primer pairs for PCR based detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, at least one primer pair per subgroup, e.g., at least one primer pair that hybridizes to one gene per subgroup; and differentiation and detection using amplification alone ("wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample.") The combination of art cited by the Examiner does not teach these elements of the claims.

Levine et al. teaches detection of five pathogenic subgroups in a sample. As the Examiner has stated, Levine et al does not teach PCR detection of all five pathogenic subgroups, and Levine et al does not teach a set of at least 5 oligonucleotide primer pairs. Lang et al. does

not remedy this deficiency of Levine et al. Lang et al teaches a PCR based method to detect and differentiate between only 2 pathogenic subgroups. The Examiner stated that

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine et al. so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang et al. in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang et al. in order to have provided a larger quantity of target DNA for the probes. Lang et al. teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method. Thus, the claimed sets of oligonucleotide primers and probes would have been prima facie obvious in light of the teachings of Levine et al. in view of Lang et al.

Thus, although Lang combined with Levine might arguably teach a method for detection and differentiation of 5 subgroups using PCR and hybridization, the combination does NOT teach a method for detection and differentiation of 5 subgroups using PCR alone, e.g., the combination does not include the element of "wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample." Since the combination of Lang et al and Levine et al does teach every element of claims 30, 36, and 37, the combination cannot render the claims obvious.

Amended claims 33 and 36 both include the element of a set of oligonucleotide probes labeled at the 5' end with a fluorescent reporter dye, at the 3' end with a fluorescent quencher dye, and susceptible to 5'-3' exonuclease degradation by a polymerase. Neither Levine et al nor Lang et al include this element; the combination of art cannot render these claims obvious.

One of skill in the art would have had no expectation of success. In addition, even if Levine et al combined with Lang et al did teach each and every element of the rejected claims

(and Applicant maintains that the combination does not) one of skill would have had no expectation of success when combining the elements.

Levine et al. teaches specific DNAs exist for the five pathogenic subgroups. For the detection of 5 E. coli pathogenic subgroups, Levine et al. discloses the use of 8 different probes. Levine teaches that more than one probe is necessary to unambiguously identify certain subgroups. Lang et al teaches use of triplex PCT method for detection of 2 subgroups (EHEC and ETEC); Lang et al teaches using three different primer pairs are necessary. Thus, in combining Levine et al. and Lang et al., one of skill would use at least 8 different primer pairs for detection of E. coli from 5 pathogenic subgroups.

One of skill would have no expectation of success when using PCR with 8 different primer pairs. As is well known, PCR with a large number of primers can give unreliable results due to interference effects. Applicants are not aware of any diagnostic PCR systems known at the priority date of the present invention in which such a high number of primers was used to detect a pathogenic agent. Indeed, it is disclosed in the discussion section of Lang et al. (page 3149, 2nd paragraph) that double bands were observed in the PCR of the labile toxin gene, possibly due to a lack of specificity, supporting Applicant's argument that one of skill would have no expectation of success when combining the elements of the cited art.

Applicant has previously argued that the publication of Schmidt et al. teaches away from the claimed invention, due to Schmidt et al.'s suggestion that PCR alone is not sufficient for a diagnosis of EaggEC strains. In the Office Action, the Examiner stated that

> Schmidt et al.'s actual quotation is "The PCR technique used here enables a more rapid diagnosis of EAggEC than other techniques currently available. However, the EAggEC probe or the adherence test must still supplement the PCR to identify the disease causing strains (sentence bridging p. 704-705)." In fact, this is not a teaching away from the use of PCR for the identification of the subgroups of pathogenic E. coli, but a clear teaching to do so, albeit in combination with other techniques. Applicant's claims are drawn using the open claim language "comprising" which

> implies that any method which utilizes the recited steps is encompassed within the claims, even if additional steps are used in the prior art method. Thus the invention to which applicant refers in the arguments "oligonucleotide primers and proves for the detection and differentiation of all known E. coli strains by PCR alone" is not claimed. Furthermore, it is noted that such a claim would require further search and consideration beyond the instantly claimed invention, or any claims which have been previously set forth in the prosecution of this application.

Applicant notes that the amended claims are drawn to methods for detection of 5 pathogenic subgroups and primer pairs useful for said methods, using PCR alone: "wherein the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample." Applicant maintains that Schmidt et al supports Applicant's contention that one of skill in the art would have no expectation of success when combining Levine et al and Lang et al.

Claim 31 is rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Levine et al. in view of Lang et al. as previously applied to claims 30, 33, 36, and 37, and further in view of Savarino et al. (PNAS USA, Vol. 90, pp. 3093-3097 (1993)). Amended Claim 31 is dependent on amended Claim 30, and is directed to a set of at least 5 oligonucleotide primer pairs for PCR (alone) based detection and differentiation of 5 subgroups of pathogenic enterobacteria in a sample, at least one primer pair per subgroup, reciting a list of specific genes in the alternative to which the primer pairs can hybridize. The combination of art cited by the Examiner does not teach the limitation of a set of at least 5 primer pairs, e.g., at least one primer pair per subgroup. As discussed above, the combination of Levine et al and Lang et al does not teach the limitation of at least 5 primer pairs, one for each subgroup. In addition, as discussed above, one of skill in the art would have no expectation of success when combining Levine et al and Lang et al. Savarino et al does not remedy the deficiencies of the combination of Levine et al and Lang et al. Accordingly the combination of Savarino et al, Levine et al and Lang et al does teach every element of claim 31 and the combination cannot render the claim obvious.

Claim 34 is rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Levine et al. in view of both Savarino et al. (PNAS USA (1993) 90:3093-3097) and Louie et al. (Epidemiol. Infect. (1994), 112:449-461). **Claim 35 is rejected under 35 U. S.C. 103(a)** as being allegedly unpatentable over Levine et al. in view of both Savarino et al. (PNAS USA (1993) 90:3093-3097) and Louie et al. (Epidemiol. Infect. (1994), 112:449-461) as applied to claim 14 above, and further in view of all of the following: Yamamoto et al. (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733), Moseley et al. (GenBank M34916), Yamamoto et al. (Infection and Immunology (1996) 64 (4): 1441-1445), Schmidt et al. (Journal of Clinical Microbiology (1995) 33(3): 701-705), Lampel et al. (US 5041372), Franke et al. (Journal of Clinical Microbiology (1994) 32(10):2460-2463), Kaper (1995, GenBank Accession Z11541), Paton et al. (GenBank Z36899), and Paton et al. (GenBank L11079).

Both Claims 34 and 35 ultimately depend on amended Claim 33, and are directed to sets of oligonucleotide probes useful for Real Time PCR, labeled at the 5' end with a fluorescent reporter dye, at the 3' end with a fluorescent quencher dye, and susceptible to 5'-3' exonuclease degradation by a polymerase. None of the art cited by the Examiner includes, at least, the element of probes useful for Real Time PCR, e.g., labeled at the 5' end with a fluorescent reporter dye, at the 3' end with a fluorescent quencher dye, and susceptible to 5'-3' exonuclease degradation by a polymerase. Therefore the combination of art does not include all elements of the claims and cannot render the claims obvious.

Claims 39 and 40 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Levine et al. in view of Lang et al. in view of Tsen et al. (Journal of Food Protection (1996) Vol. 59, No. 8, pp. 795-802). Claims 39 and 40 both ultimately depend on Claim 21, and are directed to PCR based methods for detection of 5 subgroups of pathogenic enterobacteria in a sample using at least 5 primer pairs, at least one primer pair for each subgroup, wherein detection of amplified product indicates the presence of at least one subgroup in the sample, wherein the method is used to detect contamination of a consumable (claim 39) and, wherein the consumable is a meat, milk, or vegetable (claim 40). The combination of art cited by the Examiner does not

include all of the elements of claims 39 and 40: as noted above, neither Levine et al not Lang et al include simultaneous detection of 5 subgroups using a set of at least 5 primer pairs with at least one primer pair for each subgroup. Tsen et al, teaching detection of the LT gene in milk, does not remedy this deficiency. Therefore the combination of art does not include all the elements of the claims and cannot render the claims obvious.

In summary, the combination of cited prior art references do not teach all of the elements of the claims and cannot render the claims obvious. Withdrawal of this ground of rejection of the claims is respectfully requested.

CONCLUSION

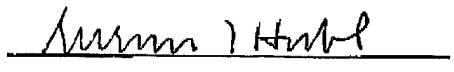
Withdrawal of the pending rejections and reconsideration of the claims are respectfully requested, and a notice of allowance is earnestly solicited. If the Examiner has any questions concerning this Response, the Examiner is respectfully requested to telephone Applicant's representative at (415) 393-2654.

NOTICE OF FIRM NAME CHANGE

Agent for Applicant wishes to inform the Office that the name of its firm has been changed to Bingham McCutchen LLP.

Respectfully submitted,

Dated:

By: 

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Appendix I

Version of the Amendments to the Claims with Markings to Show Changes Made

21. (Amended) A ~~Polymerase Chain Reaction (PCR)~~ method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

isolating nucleic acid from said sample;

adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least ~~one~~ five oligonucleotide primer ~~pair~~ pairs, wherein at least one primer pair is capable of specifically amplifying a DNA sequence to produce an amplified product of a virulence factor/toxin gene characteristic for each one of ~~the subgroups of pathogenic E. coli, said subgroups comprising~~ an enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic ~~and~~ or enterohemorrhagic pathogenic E. coli strains;

subjecting said sample and said set of primer pairs to an amplification process ~~to produce amplified product~~; and

detecting the presence of at least one amplified product, wherein the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample.

22. (Amended) The method according to claim 21, wherein the set of oligonucleotide primer pairs comprises primer pairs selected from the group consisting of:

~~a~~ at least one primer pair that hybridizes to a gene encoding heat labile toxin or a gene encoding heat stabile toxin for amplification of a DNA sequence characteristic for enterotoxigenic E. coli;

~~a~~ at least one primer pair that hybridizes to a gene encoding heat stabile toxin or to a pCDVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative E. coli;

~~a primer pair that hybridizes to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative E. coli;~~

~~a~~ at least one primer pair that hybridizes to ~~the~~ a inv-plasmid for amplification of a DNA sequence ~~contained in~~ characteristic for enteroinvasive E. coli;

~~a~~ at least one primer pair that hybridizes to ~~the~~ a EAF plasmid, or ~~the~~ an eae gene for amplification of a DNA sequence characteristic for enteropathogenic E. coli; and

~~a~~ at least one primer pair that hybridizes to ~~the~~ genes encoding shiga-like toxin sltI or sltII for amplification of a DNA sequence characteristic for enterohemorrhagic E. coli.

24. (Amended) The method according to claim 21, ~~further comprising~~ wherein detecting the presence of at least one amplified product is performed using ~~an~~ at least one oligonucleotide probe capable of hybridizing to ~~a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic E. coli, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and~~ the amplified product wherein said oligonucleotide probe is labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and is susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein said amplification process uses a polymerase having 5'-3' exonuclease degradation activity.

25 (Cancelled) ~~The method according to claim 24 wherein the labeled oligonucleotide probe is specific for the respective virulence factor/toxin gene to be detected.~~

26. (Amended) The method according to claim ~~25~~ 24 wherein the labeled oligonucleotide probe is selected from the group consisting of:

a labeled oligonucleotide probe specific for the detection of a heat labile toxin gene characteristic for enterotoxigenic E. Coli;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of a heat stabile toxin gene characteristic for enterotoxigenic E. Coli;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of a heat stabile toxin gene characteristic for enteroaggregative E. Coli;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of a pCVD432 plasmid;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of ~~the~~ a inv-plasmid;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of ~~the~~ a EAF-plasmid;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of ~~the~~ a eae gene;

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of a shiga-like toxin SltI gene; and

~~the~~ a labeled oligonucleotide probe ~~is~~ specific for the detection of a shiga-like toxin SltII gene.

29. (Amended) The method according to claim 21 wherein the amplification process comprises 35 PCR cycles at a $MgCl_2$ concentration of 5.2 ~~mmol~~ mM, an annealing temperature of 55 °C and an extension temperature of 65 °C.

30. (Amended) A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of pathogenic enterobacteria in a sample wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample, wherein said set comprises at least five primer pairs, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide primer pairs.

31. (Amended) The set of primer pairs according to claim 30 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or to a gene encoding heat stabile toxin of enterotoxigenic E. coli;

a primer pair that hybridizes to a gene encoding heat stabile toxin or to a pCVD432 plasmid of enteroaggregative E. coli;

~~a primer pair that hybridizes to the pCVD432 plasmid of enteroaggregative E. coli;~~

a primer pair that hybridizes to ~~the~~ a inv-plasmid of enteroinvasive E. coli;

a primer pair that hybridizes to ~~the~~ a EAF plasmid, or ~~the~~ a eae gene of enteropathogenic E. coli; and

a primer pair that hybridizes to ~~the~~ a gene encoding shiga-like toxin sltI or sltII of enterohemorrhagic E. coli.

33. (Amended) A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real Time–PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic E. coli strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains, wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and are susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein for detection and differentiation of each subgroup at least one probe is included in the set of oligonucleotide probes.

34. (Amended) The set of probes according to claim 33 comprising:

a labeled oligonucleotide probe specific for the detection of heat labile toxin gene characteristic for enterotoxigenic E. Coli or a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enteroaggregative E. Coli or a labeled oligonucleotide probe specific for the detection of pCVD432 plasmid;

a labeled oligonucleotide probe specific for the detection of the inv-plasmid;

a labeled oligonucleotide probe specific for the detection of the EAF-plasmid or a labeled oligonucleotide probe specific for the detection of the eae gene; and

a labeled oligonucleotide probe specific for the detection of shiga-like toxin SltI gene or ~~and~~ a labeled oligonucleotide probe specific for the detection of shiga-like toxin SltII gene.

36. (Amended) A set of oligonucleotide primer pairs and a set of oligonucleotide primer probes useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real time PCR method, wherein said ~~set~~ sets of oligonucleotide primer pairs and oligonucleotide primer probes ~~allows~~ allow detection and differentiation of pathogenic enterobacteria in a sample, wherein said set of oligonucleotide primer pairs comprises at least five primer pairs and ~~each~~ at least one primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for ~~one~~ each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains ~~and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide probes and a set of oligonucleotide probes,~~ and, wherein said set of oligonucleotide probes comprises at least one oligonucleotide probe, each oligonucleotide probe specifically binding a sequence of a ~~detect~~ virulence factor/toxin genes characteristic of one of the subgroups of pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains by real time PCR.

38. (Amended) The method of claim ~~27~~ 37, wherein said sample is derived from a human.

40. (Amended) The method of claim ~~29~~ 39, wherein said consumable is selected from the group consisting of meat, milk ~~or~~ and vegetable.